TOPICAL HYPOSENSITIZATION OF ALLERGIC CONTACT SENSITIVITY IN THE GUINEA PIG*

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When an adequate amount of an antigenic chemical such as dinitrochlorobenzene (DNCB) is applied to the skin of a guinea pig, an allergic contact sensitivity to that compound may result; this sensitivity is analogous to contact sensitivity in man. After the appearance of sensitization, however, further application of the same chemical to the skin may eventually produce a specific depression of sensitivity to the compound. This second phenomenon is known as topical hyposensitization; the term "hardening" is sometimes used to refer to the same phenomenon, particularly when the sensitivity is occupational.

Topical hyposensitization with poison ivy antigen (pentadecylcatechol) has been produced experimentally in man by Kligman (1). In the guinea pig it has been reported by Kobayashi (2), Landsteiner and Chase (3), and Jadassohn, Brun, and Bujard (4). None of these experimental studies was controlled to rule out the possibility that non-specific effects of prolonged administration of antigen and of the resulting inflammation may have produced a general lowering of sensitivity to all antigens, rather than specific hyposensitization.

The following studies were designed, first of all, to study the phenomenon of topical hyposensitization under controlled circumstances, and to verify its existence. An additional goal was to describe some of the prominent features of this phenomenon in an attempt to achieve some knowledge of its usefulness and with the hope of gaining some insight into its underlying mechanisms.

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This investigation was supported in whole by Public Health Service Research Grant No. AI-04935-02, from the Division of Research Grants, National Institutes of Health.

This research was performed during tenure of U.S.P.H.S. post-doctoral fellowship (GPD 14, 485 C1).

Presented at the Twenty-fifth Annual Meeting of The Society for Investigative Dermatology, Inc., San Francisco, Calif., June 22, 1964.

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METHODS

Subjects were young adult male albino guinea pigs, weighing 250–400 grams at the start of the experiment.

Sensitization: Animals were sensitized to Taractan (chlorprothixene, a thioxanthene derivative used as a "tranquilizer") by one or two intradermal injections of 1.2 mg of the compound in aqueous solution prepared for injection.‡ Sensitization to DNCB was accomplished by a single application of 1 mg DNCB in acetonic solution to the wax-epilated skin of the nape. These two sensitization procedures were carried out several days apart, and animals were sensitized to DNCB last, in order to avoid interference phenomena.

Hyposensitization procedure: In studies of the effects of repeated applications of antigen on sensitivity, the antigen was dissolved in 0.1–0.2 ml of the appropriate solvent and evenly applied to an area approximately 5 × 3 cm on the right side of the animal. This area was wax-epilated at weekly intervals, or more often when necessary. Hyposensitizing applications were made daily for 5 days a week.

Testing: For skin tests the antigen was applied in the appropriate solvent to an area 1 cm in diameter on skin that had been wax-epilated 48 hours before. Sensitivity to a given antigen was tested with at least 2 such tests. As a rule, 4 such tests were made at one time, 2 to each of 2 antigens. These 4 tests were placed at the corners of an imaginary quadrangle on the side of the animal so that the 2 tests with a given antigen were diagonally opposite to each other. By an unusual coincidence, it was convenient to use 10 γ and 25 γ of both antigens for test pairs. These doses evoked no reaction in the unsensitized animal.

The tests were read at 12, 24, and 36 hours. Each test was graded on the following scale:

0. No reaction.
0.5 Faint erythema.
1.0 Erythema of part of the test area.
2.0 Erythema of the whole area, or partial erythema with induration.
3.0 Erythema and induration of the entire test area.

As the tests were always given in pairs, the data were simplified by adding the two test scores for tabulation. This yielded an "intensity of reaction" score for the antigen which could be as low as 0 or as high as 6.0 (*e.g.*, two 3.0 tests).

‡ Taractan was generously supplied by Edward Miller, M.D., of Hoffmann-La Roche, Inc. We were fortunate in having a group of animals which was very susceptible to sensitization with Taractan; the sensitizing potency of this compound is variable.

Experimental design: Thirty-two guinea pigs were sensitized to both DNCB and Taractan, given a preliminary test to verify sensitization, and divided into the following three groups:

Group 1 (9 animals): Control animals subjected only to sensitization and testing.

Group 2 (17 animals): Taractan, 100–1,000 γ was applied to the wax-epilated skin of one side 5 days a week for 10 weeks. Animals receiving different amounts (100 γ, 250 γ, and 1,000 γ) of Taractan were combined into this group as they showed identical trends.

Group 3 (6 animals): DNCB, 250 γ, was applied in the same way.

In all animals, sensitivity to both antigens was measured by skin tests placed on the untreated side at several points during the course of the experiment; the first test was conducted after 2 weeks of treatment. In addition, identical tests were placed on both sides, treated and untreated, after the sixth week of treatment.

An important feature of the design of these experiments is that the subjects were sensitized to 2 antigens, and that each of the 2 antigens was used to produce hyposensitivity in one group. Experiments in which "specific" depression is produced by one antigen, while the other antigen always serves as control may lead to error. A variety of non-specific factors may selectively depress the reaction to one of two antigens, either because one produces a "weaker" sensitivity than the other, or because of differences in the sensitivity of the testing methods. We have found, for instance that the reaction to NDMA (p-nitrosodimethylaniline) is more readily suppressed by non-specific agents than is that to DNCB.

RESULTS

Specific hyposensitization was produced by both DNCB and Taractan within the first 2 weeks of treatment. This is illustrated in Figures 1 and 2. It must be noticed that in Figure 1 the intensities of test reactions to Taractan are presented; Figure 2 presents the results of tests with DNCB, conducted at the same times in the same animals. Both figures are based on readings made 36 hours after application of tests.

All but 2 of the 17 animals treated with Taractan had total sensitivity scores between 0 and 2 when tested after 2 weeks' treatment, while all but 2 of the 9 control animals had scores in the 4 to 6 range. Statistical analyses show that the specific depression produced by Taractan was unquestionable. Although only 6 animals were given DNCB, a nonparametric test (Mann-Whitney U test) shows that after 2 weeks' treatment the DNCB reaction was significantly depressed compared to that seen in controls ($P = .015$).

Another experiment of identical design was conducted using DNCB and p-nitrosodimethylaniline (NDMA) as antigens. This experiment, which involved 42 animals, also showed specific depression of the reaction to whichever antigen was applied daily.

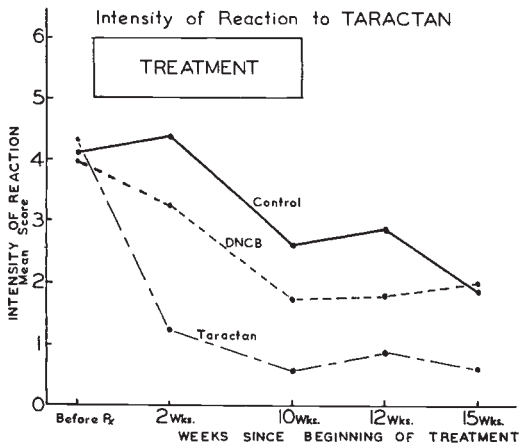

FIG. 1.

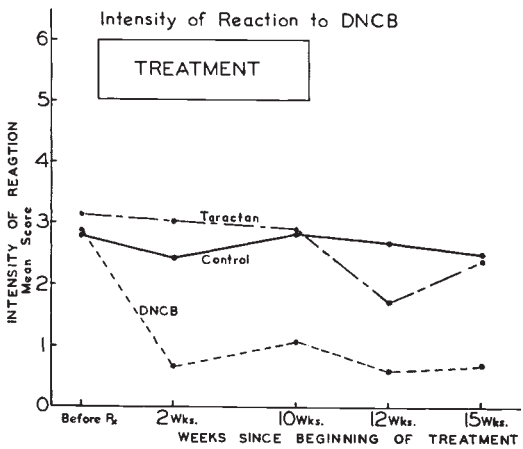

FIG. 2.

FIG. 1. The mean intensities of test reactions to Taractan are presented for the 3 groups of animals: control animals, animals treated with DNCB, and animals treated with Taractan. Results of tests with DNCB, conducted simultaneously, are presented in the other figure.

FIG. 2. The mean intensities of test reactions to DNCB are presented for the 3 groups of animals; controls, animals treated with Taractan, and those treated with DNCB. The animals were always tested with Taractan simultaneously, and results of Taractan tests are presented in Figure 1.

Variability among antigens: Although hyposensitization was always grossly apparent with Taractan and NDMA, it was much more difficult to achieve with DNCB. With this antigen, an occasional animal would always fail to show the phenomenon, and the degree of depression seen was less. When more than 1 mg a day was given, non-specific depression of the reaction to both antigens often appeared; with less than this, hyposensitization might fail to appear.

Inflammation at the site of treatment: When DNCB or NDMA was applied daily to the skin of a sensitized guinea pig, moderate to severe inflammation developed which persisted as long as the compound was applied. Although the dose given was far below the irritant range, this inflammation may have been due in part to a cumulative irritant effect. When Taractan (which is not an irritant to the skin surface) was used for hyposensitization, a moderate inflammation developed which reached its height after about 2 weeks of treatment and then slowly decreased. The area treated with Taractan was usually normal in appearance after 6–8 weeks' treatment. It is convenient to think of the process of hyposensitization as a neutralization reaction which takes place primarily where the antigen is applied. A great deal of visible inflammation results when the sensitivity is strong, when the area treated is small, and when a large amount of antigen is given at one time. If the sensitivity is moderate, if the area is large, and if the neutralization process is extended in time, less inflammation will be seen. This point was illustrated by another experiment in which hyposensitization to Taractan was carried out by application to the wax-epilated side of the animal of gradually increasing doses of antigen, beginning with 25 γ a day, and reaching 200–400 γ after 3 weeks. Only animals of moderate initial sensitivity were included in the study. Under these circumstances, the amount of local inflammation was reduced to an intermittent erythema, only slightly redder than that seen in control animals who were repeatedly wax-epilated, but not hyposensitized.

"Local desensitization": On several occasions in these experiments, animals were tested simultaneously on both sides. This permits comparison of the degree of hyposensitization achieved in the distant skin and in the area of daily application of the antigen. Although the treated area of skin tended to be more reactive to certain compounds, making the difference between positive and negative tests more striking, no valid evidence for "local desensitization", such as was hypothesized by Hunziker (5), was found.

Duration of depressed sensitivity: Although the hyposensitizing procedure was stopped after 10 weeks in the experiment described, specific depression of sensitivity was still obvious 5 weeks later (see Figures 1 and 2). In another experiment, hyposensitization persisted for 2 months, at which time the experiment was terminated.

At the end of the 5 week observation period described above (15 weeks after hyposensitization was begun), all animals were given an injection of 1.2 mg of Taractan emulsified in Freund's adjuvant into one rear foot pad. Although this was a more drastic procedure than that originally used for sensitization, it did not restore—or even increase—sensitivity to Taractan in the hyposensitized animals. Two weeks after the injection of Taractan described above, the same animals were given 1 mg DNCB in Freund's adjuvant by the same route; again no recovery of sensitivity was seen in the hyposensitized animals. When the same animals were later given 1 mg of NDMA (an antigen to which they had not been previously sensitized) in the same way, brisk sensitization occurred; this suggests that the failure of the hyposensitized animals to respond to further sensitization procedures was due to their prior experience with these antigens, and cannot be attributed to a generalized deterioration of their immunologic machinery.

Attempts to prevent the appearance of sensitivity: The mechanisms which underlie the phenomena of topical hyposensitization are unknown, but one simple hypothesis which deserves consideration is that the applied antigen in some quantitative way "neutralizes" the sensitivity of the animal. When the animal is fully sensitized to start, there is a large reservoir of sensitivity which must be neutralized by the topically applied antigen at a rate faster than that at which "new" sensitivity can be generated (for the same application of antigen that neutralizes may also have a sensitizing effect).

If this were all there is to it, then it should be possible to prevent the appearance of a positive skin test on untreated skin by beginning the hyposensitizing treatment with the first exposure to the compound, *before* sensitivity has appeared. Under these circumstances, it should be a simple matter to neutralize the sensitivity as it appears, presumably arising from a zero level, so that although inflammation would be present at the treatment site, a positive test on normal skin would never appear.

Several such experiments were attempted, using varying amounts of both NDMA and DNCB as antigen. In a typical experiment, 500 γ of NDMA was applied every day to the skin of one group, while a control group received a single sensitizing dose of NDMA. In all experiments, the animals treated daily with antigen developed *higher* sensitivity (as measured by tests on untreated skin) than did controls; in fact, daily application of antigen is a very effective way to produce a high level of sensitization.

It is paradoxical that hyposensitization occurs at all—for the same series of exposures to antigen which sensitizes the virgin animal has the opposite effect of depressing sensitivity in the animal which has already been sensitized. Apparently the sensitization which results from a first exposure to the antigen is so great that it is virtually impossible to "neutralize" it during the first days of sensitization; while in the previously sensitized animal the same procedure produces less additional sensitization, and hyposensitization can take place. Thus, the process of contact sensitization in the guinea pig subjected to repeated applications of antigen appears to go through a phase of great activity, followed by a period of relative refractoriness to further contact sensitization.

This refractoriness may be due in part to the continued bombardment with antigen which is involved in the hyposensitization procedure, or it may simply be part of the natural course of contact sensitivity. Evidence to be presented elsewhere suggests that the latter is the case: After sensitization has been achieved the animal becomes relatively refractory to further sensitization, and this is why topical application of the antigen at that time can produce hyposensitization instead of heightening the degree of sensitivity.

SUMMARY

Specific depression of allergic contact sensitivity can be produced in the previously sensitized guinea pig by daily topical application of small amounts of the antigen over a 2 week period. This is described with 2 antigens, DNCB and Taractan, and is reported with a third (NDMA). Such hyposensitization is more readily accomplished with some sensitizers (*e.g.*, Taractan, NDMA) than with others (*e.g.*, DNCB).

During the early stages of hyposensitization, sensitivity to the compound being applied is depressed on untreated areas of skin, while inflammation occurs at the site of daily application. In the case of Taractan, inflammation at the site of application may be transient or mild.

After daily application of the antigen was stopped, specific depression of sensitivity persisted for at least 5 weeks. Sensitivity could not be restored to hyposensitized animals by sensitization procedures which are usually quite effective.

Hyposensitization procedures which effectively depress sensitivity in the sensitized animal do not prevent or delay appearance of sensitivity following initial exposure to the antigen.

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